

## Metal Chelation as a Potential Therapy for Alzheimer's Disease

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**ABSTRACT:** Alzheimer's disease is a rapidly worsening public health problem. The current lack of effective treatments for Alzheimer's disease makes it imperative to find new pharmacotherapies. At present, the treatment of symptoms includes use of acetylcholinesterase inhibitors, which enhance acetylcholine levels and improve cognitive functioning. Current reports provide evidence that the pathogenesis of Alzheimer's disease is linked to the characteristic neocortical amyloid- $\beta$  deposition, which may be mediated by abnormal metal interaction with A $\beta$  as well as metal-mediated oxidative stress. In light of these observations, we have considered the development of drugs that target abnormal metal accumulation and its adverse consequences, as well as prevention or reversal of amyloid- $\beta$  plaque formation. This paper reviews recent observations on the possible etiologic role of A $\beta$  deposition, its redox activity, and its interaction with transition metals that are enriched in the neocortex. We discuss the effects of metal chelators on these processes, list existing drugs with chelating properties, and explore the promise of this approach as a basis for medicinal chemistry in the development of novel Alzheimer's disease therapeutics.

### INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by extracellular deposits of amyloid- $\beta$  protein (A $\beta$ ), the main component of neuritic or senile and diffuse plaques.<sup>1</sup> Pathogenic mutations of the APP gene close to or within the A $\beta$  domain are linked to forms of familial AD (FAD).<sup>2</sup> Inheritance of mutations on chromosome 14 (presenilin-1),<sup>3</sup> or chromosome 1 (presenilin-2)<sup>4</sup> produces the more aggressive form of the disease (early-onset age of 25–45 years). Moreover, apolipoprotein-E (apoE)  $\epsilon$ 4 allele on chromosome 19 has been identified

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as a risk factor for late-onset AD.<sup>5</sup> More recently, a genetic deletion of the  $\alpha_2$ -macroglobulin (A2M) gene on chromosome 12 was discovered to be another risk factor for AD.<sup>6</sup>

Although the effects of the genetic lesions that cause FAD are to elevate  $A\beta_{1-42}$  levels,<sup>7</sup> the mere presence of  $A\beta_{1-42}$  cannot initiate amyloid deposition since the peptide is a normal component of healthy CSF.<sup>8</sup> If elevated cortical  $A\beta$  concentrations were solely responsible for the initiation of amyloid, it would be difficult to explain why the amyloid deposits are focal (related to synapses and the cerebrovascular lamina media) and not uniform in their distribution. Importantly, overexpression of  $A\beta_{1-42}$  from birth, which occurs in genetic forms of AD (FAD and Down's syndrome), does not induce amyloid deposition in childhood.<sup>9</sup> In these cases,  $A\beta$  deposition still occurs in an age-dependent, albeit accelerated manner. Also, we have found the total levels of  $A\beta$  measured in postmortem brain tissue from AD cases are increased to the same extent in both brain regions that form abundant amyloid (e.g., hippocampus) compared to tissues that do not form amyloid (such as cerebellum).<sup>10</sup> From these observations, it seems unlikely that  $A\beta$  overproduction alone initiates  $A\beta$  deposition, and thus, it is more likely that there are neurochemical factors, altered as a stochastic consequence of aging, that initiate  $A\beta$  deposition in sporadic AD and FAD. The plaque deposits of  $A\beta$  appear then to be a morphological variation of  $A\beta$  accumulation caused by neurochemical interactions that are specific to the neocortex. The availability of high concentrations of Cu(II) and Zn(II) is a specific feature of neocortical tissue that could explain the condensation of  $A\beta$  as plaque. Here, we review current evidence for abnormal metal interactions in AD and discuss the potential therapeutic effect of metal chelators against AD pathology.

### CEREBRAL ZINC, COPPER AND IRON LEVELS IN AD

There is an emerging consensus in the literature to indicate that the homeostases of zinc, copper, and iron are significantly altered in the AD brain tissue (reviewed in Refs. 11,12). For example, abnormal levels of zinc, copper, or iron have been found in several subcortical regions such as the hippocampus, amygdala, and olfactory bulb, as well as the neocortex.<sup>11,12</sup> A recent well-controlled study using microparticle-induced X ray emission (PIXE) analysis of the cortical and accessory basal nuclei of the amygdala indicated that zinc, copper, and iron accumulate in the neuropil and plaques of the AD brain where their concentrations are 3–5-fold increased compared to age-matched controls (TABLE 1). In fact, the concentrations of these metal ions, particularly the redox active Cu and Fe (implicated in free radical reactions)<sup>13</sup> are normally concentrated in those regions of the brain most affected by AD pathology. Evidence for abnormal Cu homeostasis in AD includes a 2.2-fold increase in the concentration of CSF Cu,<sup>14</sup> and an accompanying increase in ceruloplasmin in the brain and CSF of AD patients.<sup>15</sup> Similarly, there is an extensive literature describing abnormal levels of Fe and Fe-binding proteins in AD.<sup>16</sup> It has been demonstrated that the Fe found within the amyloid deposits of human brains and in amyloid-bearing APP transgenic mice brains is redox-active.<sup>17</sup>

**TABLE 1. Micro-PIXE analysis of metal ion concentrations in Alzheimer's disease plaque and neuropil**

	Zinc $\mu\text{g/g}$ ( $\mu\text{M}$ ) <sup>a</sup>	Copper $\mu\text{g/g}$ ( $\mu\text{M}$ ) <sup>*</sup>	Iron $\mu\text{g/g}$ ( $\mu\text{M}$ ) <sup>*</sup>
Senile plaque	69 (1055)	25 (393)	53 (940)
AD neuropil	51 (786)	19 (304)	39 (695)
Control neuropil	23 (346)	4 (69)	19 (338)

<sup>a</sup>Adapted from Ref. 18. For purposes of comparison we have converted the published values into molar concentrations assuming a sample density of 1 g/cm<sup>3</sup>.

### BIOMETALS AND RISK FACTORS FOR ALZHEIMER'S DISEASE

The metal binding proteins, A2M<sup>19</sup> and apoE,<sup>20</sup> are typically found in senile plaques.<sup>21</sup> A2M mediates A $\beta$  degradation via its low density-related lipoprotein (LRP) receptor.<sup>22</sup> A2M binding with A $\beta$ , which is enhanced by the presence of zinc, precludes A $\beta$  fibrillogenesis and reduces its associated neurotoxicity.<sup>23</sup> Meanwhile, of the three apoE isoforms, the  $\epsilon$ 4 isoform has been found to be the least effective in inhibiting Cu(II)- and Zn(II)-induced precipitation of A $\beta$ ,<sup>20</sup> a finding compatible with the apoE  $\epsilon$ 4 allele being an independent risk factor for AD.<sup>5</sup> It is interesting to note that AD patients carrying the apoE  $\epsilon$ 4 allele have been found to have elevated serum zinc and copper levels, providing an association between abnormal metal metabolism and apoE risk for AD.<sup>24</sup> Both iron and copper have been shown to enhance the toxicity of A $\beta$  in cultures,<sup>25,26</sup> and copper toxicity is enhanced by nontoxic concentrations of A $\beta$  via A $\beta$ -mediated glutathione depletion.<sup>27</sup>

### APP AND A $\beta$ ARE METAL-BINDING PROTEINS

Investigating the role of brain biometals as a potential target for therapeutic remedies in AD stemmed from a series of *in vitro* studies that found that synthetic A $\beta$  and purified APP exhibited several physicochemical interactions with Zn(II), Cu(II), and to a lesser extent, Fe(III), at low micromolar and submicromolar concentrations of the metal ions.<sup>28-34</sup> Hence, disruption in the homeostasis of these metals could possibly contribute to abnormal metal-A $\beta$  interactions in AD.

Specific and saturable binding sites for zinc (APP 181-200;  $K_a = 750$  nM)<sup>28</sup> and copper (APP 135-155;  $K_d = 10$  nM)<sup>35</sup> were identified within the cysteine-rich region on the ectodomain of APP 695. These sites have homology in all known members of the APP superfamily.<sup>31</sup> This indicates that zinc and copper interaction with the protein may play an important, evolutionary conserved role in APP function and metabolism.

While Zn(II) binding to APP is believed to have a structural role, APP can reduce Cu(II) to Cu(I), which results in oxidation of Cys-144 and Cys-155 and a corresponding intramolecular disulfide bridge formation.<sup>36</sup> The resulting APP-Cu(I) complex is prone to redox reactions that result in site-specific APP fragmentation.<sup>37</sup> The Cys-144 residue of APP was determined to be necessary for this chemical reaction.<sup>38</sup>

A $\beta$ <sub>1-40</sub> specifically and saturably binds zinc, manifesting high-affinity binding ( $K_d = 107$  nM) with a 1:1 (zinc:A $\beta$ ) stoichiometry, and low-affinity binding ( $K_d = 5.2$   $\mu$ M) with a 2:1 stoichiometry.<sup>29</sup> This binding is histidine-mediated, since it is abolished by acidic pH, and by chemical blocking of the histidine residues.<sup>34</sup> More importantly, His-13 is believed to be an important residue in zinc-mediated A $\beta$  assembly.<sup>39</sup> The zinc binding site was mapped to a stretch of contiguous residues between positions 6–28 of the A $\beta$  sequence. Occupation of the zinc binding site, which straddles the lysine 16 position of  $\alpha$ -secretase site, inhibits  $\alpha$ -secretase type (tryptic) cleavage and so may influence the generation of A $\beta$  from APP, and may increase the biological half-life of A $\beta$  by protecting the peptide from proteolytic attack.<sup>30</sup> Zinc concentrations above 300 nM rapidly precipitate synthetic human A $\beta$ <sub>1-40</sub>.<sup>29,32,33</sup> Interestingly, zinc may also preserve the  $\alpha$ -helical conformation of A $\beta$ <sub>1-40</sub>, which may explain why precipitation of A $\beta$  by Zn(II) is reversible, and that Zn(II)-assembled A $\beta$  can be resolubilized by chelation.<sup>33</sup>

A $\beta$  is also precipitated by Cu(II) in a reaction that is potentiated by mildly acidic (pH 6.6) conditions. The stoichiometry of Cu:A $\beta$  increases from zero when A $\beta$  is soluble to 1.0–2.5 when A $\beta$  is aggregated by Cu(II).<sup>34,40</sup> A $\beta$ <sub>1-40</sub> has higher-affinity (log  $K_{app}$  10) and lower-affinity (log  $K_{app}$  7.0) binding sites for Cu, but the affinity of Cu for A $\beta$ <sub>1-42</sub> is greater for both sites (log  $K_{app}$  17.3 and log  $K_{app}$  8.0, respectively).<sup>40</sup> The high-affinity Cu(II) binding site on A $\beta$ <sub>1-42</sub> is of such high affinity that it is very likely to be occupied *in vivo*. However, under mildly acidic conditions (pH 6.8), the affinity of A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> for Zn(II), but not Cu(II) decreases at the lower-affinity binding sites.<sup>40</sup>

Unlike Zn(II), both Cu(II) and Fe(III) induce greater A $\beta$  aggregation under mildly acidic conditions (e.g., pH 6.8–7.0).<sup>32,34</sup> However, A $\beta$  binds equimolar amounts of Cu(II) and Zn(II) at pH 7.4, while Cu(II) displaces Zn(II) from Zn(II):A $\beta$  aggregates under acidic conditions (pH 6.6).<sup>40</sup> A $\beta$ <sub>1-42</sub> markedly aggregates in the presence of trace amounts (<0.1  $\mu$ M) of Cu(II).

### OXIDATIVE MECHANISMS OF A $\beta$ NEUROTOXICITY

Both A $\beta$  deposition in the neocortex, and oxidative stress, are considered closely related to the pathogenesis of AD. The deposition of A $\beta$  in the neocortex of APP transgenic mice overexpressing A $\beta$  is accompanied by some neuropathological features of AD such as neuronal loss,<sup>41</sup> and signs of oxidative damage<sup>42</sup> suggesting that the neurotoxic events of AD are seminally related to A $\beta$  accumulation. Many studies have now confirmed that A $\beta$  is neurotoxic in cell culture<sup>43</sup> and *in vivo*.<sup>44</sup> Therefore, prevention of A $\beta$  deposition could be a therapeutic target in AD.

Focus on the pathogenic relevance of oxidative stress in AD was stimulated by the report that treatment of AD subjects with the antioxidant vitamin E delays de-

cline in independent functioning.<sup>45</sup> Metabolic signs of oxidative stress such as oxygen radical-mediated damage of brain proteins, lipids, and nucleic acids, as well as systemic signs of oxidative stress and the response of antioxidant systems have all been observed in AD.<sup>46,47</sup> The biochemical relationship between A $\beta$  deposition in AD and oxidative stress is complex, and the mechanisms underlying the association between oxidation and amyloid deposition are not well understood.

Synthetic A $\beta$  peptides exert toxicity through mechanisms that involve the generation of cellular hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).<sup>48</sup> The observed cytotoxicity is abolished by O<sub>2</sub><sup>-•</sup>/H<sub>2</sub>O<sub>2</sub> scavengers.<sup>49</sup> Taken together, the chemical nature of oxidation stress in AD indicates that H<sub>2</sub>O<sub>2</sub> levels may be elevated in the AD brain. While Zn(II) is redox-inert, we recently reported that the binding of trace concentrations of redox-active metals Cu(II) and Fe(III) to A $\beta$  engenders the cell-free catalytic production of H<sub>2</sub>O<sub>2</sub> from O<sub>2</sub> via metal reduction.<sup>50</sup> The redox activities of A $\beta$  species are greatest for A $\beta$ <sub>42</sub>>A $\beta$ <sub>40</sub>>>rat A $\beta$ <sub>40</sub>, a chemical relationship that corresponds to the participation of the respective peptide in amyloid pathology, as well as the peptide's H<sub>2</sub>O<sub>2</sub>-mediated toxicity in cell culture.<sup>26</sup> It is interesting to note that the A $\beta$  peptide has a selective vulnerability to Cu-mediated OH<sup>•</sup> attack that oxidizes the peptide, emulating the chemical changes seen in the A $\beta$  extracted from AD brain (Atwood *et al.* submitted).

#### METAL COMPLEXING PROPERTIES OF CHELATING AGENTS: AN OVERVIEW

The term chelator originated from the Greek word "chele," which means "crab's claw."<sup>51,52</sup> This term defines the complexes formed by a ligand (a molecule with at least two donor groups or coordination number) with their substrates (ions) such that a "ring" system is established. The process of creating such a ring structure is well correlated with the formation of a more stable complex.<sup>52</sup> Denticity (from the word "dens," meaning tooth) is used to describe the number of available donor groups of a chelating agent to bind metal ions.<sup>52</sup> For example, bidentate refers to two donor groups, tridentate to three, quinquidentate to five, and so forth. Some chelators are able to form multidentate complexes, while others can only attain a monodentate or bidentate chelate rings. For multidentate ligands, the dissociation constant ( $K_d$ ; a constant that reflects the intrinsic strength of metal-ligand binding) can vary markedly for different species.<sup>51</sup> Some chelators have the ability to directly permeate cell membranes prior to or upon binding metal ions (e.g., see Ref. 53). Other chelators become membrane permeable after esterification, or by acquiring a nonpolar state following metal complexation.<sup>51</sup> In addition, some chelators are ionophores since their chelating sites have limited flexibility and thus would prefer cations that fit easily into their molecular structure.<sup>52</sup> Ionophores may selectively enhance the permeability of metal ions in lipid membranes of cells as in the case of calcium ionophore A23187 (calcimycin), which facilitates entry of calcium ions into cells.<sup>54</sup> Similarly, pyrithione is a zinc chelator that neutralizes zinc neurotoxicity, but also has an ionophoric property.<sup>53</sup> Hence chelators may act to either deprive biological systems of metal ions, or may have the opposite effect of promoting metal uptake into cells.

## EFFECTS OF METAL CHELATORS ON A $\beta$ PATHOPHYSIOLOGY

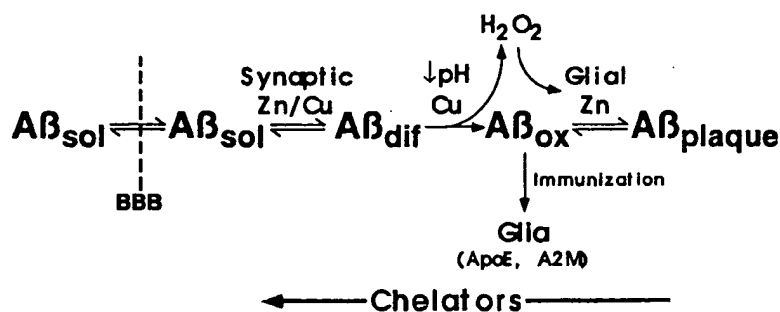
### *Inhibition of the Redox Activity and Aggregation of A $\beta$*

FAD-linked mutations of APP, presenilin-1 and presenilin-2, increase both A $\beta$  amyloid burden and A $\beta_{1-42}$  production, underscoring the role that the longer A $\beta$  species plays in AD pathogenesis.<sup>7</sup> Hence, much effort has focused on the mechanisms of A $\beta_{1-42}$ -mediated fibrillogenesis. A landmark *in vitro* study in this area was that of Jarrett *et al.*<sup>55</sup> who reported that A $\beta_{1-40}$ , which is kinetically stable at 20  $\mu$ M in solution for nine days, is destabilized over that interval by "seeding" with 2  $\mu$ M A $\beta_{1-42}$  fibrils. This work has been considerably augmented and has led to a theory of nucleation-dependence or "seeding" of amyloidogenic peptides in several neurodegenerative diseases. We extended the work of Jarrett *et al.*<sup>55</sup> and found that addition of Zn(II), Cu(II), and Fe(III) enhanced A $\beta_{1-42}$ -initiated seeding of A $\beta_{1-40}$  (Huang *et al.* submitted). However, we measured the concentrations of these metal ions that contaminate the incubation buffer and found levels to be 0.1–0.5  $\mu$ M, great enough to precipitate A $\beta$ . Therefore, we tested the effects of chelators upon this classic nucleation reaction, and found that polyamincarboxylic acid compounds like diethylene tetraamine pentaacetic acid (DTPA), and cyclohexane diamine tetraacetic acid (CDTA), both high-affinity Cu(II) and Zn(II) chelators, abolished the seeding reaction. Furthermore, NMR spectroscopy proved that these compounds do not interact directly with the peptide. These findings indicate that metal ions are essential for the initiation of nucleation-dependent fibrillogenesis.

We have shown that A $\beta$  reduces Cu and Fe, and that A $\beta$  can generate H<sub>2</sub>O<sub>2</sub> through a metal-dependent reaction.<sup>50</sup> We thus tested the possibility that metal chelators could interfere with the redox activity of A $\beta$ . We observed that BP and DTPA abolished (at a 200-fold molar excess) the H<sub>2</sub>O<sub>2</sub> generated by A $\beta$  (10  $\mu$ M) interacting with Fe(III)-citrate (1  $\mu$ M).<sup>50</sup> We repeated this experiment using a 200-molar excess of desferrioxamine (DFO, a high-affinity Fe(III) chelator), and found that DFO was ineffective in preventing H<sub>2</sub>O<sub>2</sub> formation unlike BP and DTPA.<sup>56</sup> These results suggest that the ability of a compound to inhibit A $\beta$ :metal-mediated redox activity is not simply a product of its affinity, but that other factors such as stereochemistry of the metal binding site, play important roles.

### *Resolubilization of A $\beta$ Plaques in Vitro and in Postmortem Human and APP Transgenic Mouse Brain Tissue*

We have shown *in vitro* that zinc-induced<sup>33</sup> or copper-induced<sup>34</sup> precipitation of A $\beta$  peptide is a chelation-reversible event. Interestingly, zinc-precipitated A $\beta$  is denser and less easily resolubilized than copper-induced precipitates.<sup>20</sup> Indeed, A $\beta$  is resolubilized and extracted from postmortem AD and non-AD control brains using metal chelators.<sup>57</sup> High-affinity Cu/Fe/Zn chelators such as N,N,N',N'-tetrakis-(2-pyridylmethyl)-ethylenediamine (TPEN) and bathocuproine disulfonic acid (BC), markedly enhanced the resolubilization of A $\beta$  deposits from postmortem AD and non-AD brain samples.<sup>57</sup> The observed increase in extractable A $\beta$  correlated with significant depletion in zinc (30%) and to a lesser extent, copper, in each of the AD cases examined ( $n = 10$ ) when compared with PBS-alone treated tissue.<sup>57</sup> The ability



**FIGURE 1.** A model for the role of metal/A $\beta$  interaction in AD. A $\beta$  is a normal soluble constituent of biological fluids including plasma and brain interstitial fluids (A $\beta_{sol}$ ). During epochs of metabolic stress (e.g., head injury, hypoxia, etc.), the uptake of zinc and copper via energy-dependent uptake mechanisms after they are released into the synapse is inhibited. This raises the concentration of zinc and copper in the synaptic vicinity, precipitating A $\beta$  at that site as diffuse amyloid deposits on histology (A $\beta_{dif}$ ). These deposits would ordinarily resolve as the metabolic stress diminishes and the interstitial zinc and copper concentrations decrease to normal again. If on the other hand, the concentration of Cu(II) remains high then Cu(II) may displace Zn(II) from A $\beta$  (especially under acidotic conditions)<sup>34</sup> abnormally generating H<sub>2</sub>O<sub>2</sub> and oxidizing A $\beta$  (carbonyl modification). Soluble oxidized forms of A $\beta$  (A $\beta_{ox}$ ) are protease resistant,<sup>64</sup> and therefore abnormally long-lived in the cortical interstitium where they drive up the H<sub>2</sub>O<sub>2</sub> levels even more, taxing the cellular scavenging mechanisms (e.g., glutathione), and contributing to neurotoxicity.<sup>25-27</sup> The elevated H<sub>2</sub>O<sub>2</sub>, being freely permeable, crosses the membrane of the neighboring glia and reacts with metallothionein, causing the liberation of the metallothionein-held pool of Zn(II).<sup>11,65</sup> The Zn(II) liberated into the interstitium by the glia precipitates the oxidized forms of A $\beta$  into plaque deposits that, as a result, have very high (~1 mM) levels of Zn(II)<sup>18,66,67</sup> but no longer produce H<sub>2</sub>O<sub>2</sub> so that plaque deposits become sites of decreased oxidative damage to neighboring tissue.

The plaque contains oxidized protease-resistant A $\beta$  that is cemented together by Zn(II), which induces even further resistance to proteolysis,<sup>30</sup> so generating a deposit that defies clearance until disaggregated by a chelator.<sup>57,58</sup> Treatment with a compound that complexes both Zn(II) and Cu(II) promotes the dissolution of A $\beta$  plaque and diffuse deposits<sup>57,58</sup> while simultaneously shutting down H<sub>2</sub>O<sub>2</sub> production,<sup>26,50</sup> and facilitating the clearance of damaged forms of A $\beta$  by the glia. Damaged/oxidized forms of A $\beta$  may be antigenically foreign, and cleared by activated glia, perhaps explaining how immunization of transgenic animals with synthetic A $\beta$  may work to clear A $\beta$  by promoting glial recognition of oxidized A $\beta$  forms.<sup>68</sup>

Synaptic zinc is more likely than copper to be responsible for the initial precipitation of A $\beta$  as diffuse deposits, because: (a) Zn(II) induces the precipitation of A $\beta$  far more extensively at pH 7.4 *in vitro* than does Cu(II);<sup>30,34</sup> (b) the concentration of synaptic Zn(II) reached during neurotransmission is 10–20-fold higher than that reached by Cu(II);<sup>69,70</sup> and (c) the distribution of vesicular (synaptically released) Zn(II) in the brain corresponds to the sites of the brain that are most prone to amyloid pathology—the cerebral cortex, and not the subcortical gray matter or the cerebellum.<sup>69</sup>

of a chelator to extract A $\beta$  depended upon the presence of Mg(II) and Ca(II), hence the chelating compound needed must be more selective for Zn(II) and Cu(II), than Ca(II) and Mg(II). Higher concentrations of Cu/Zn chelator caused a paradoxical decrease in the amount of A $\beta$  released, because the sequestration of Ca/Mg from the sample became substantial. This work was further extended on APP transgenic

"Hsiao" mouse brain samples using either triethylenetetraamine (TETA; a high-affinity Cu(II) chelator) or bicinehoninic acid (BCA; a Cu(I)-selective chelator) giving similar results to that of the human study.<sup>58</sup>

#### *The Chelating Class of Molecule as a Pharmacological Possibility in AD*

Presently, the only AD therapeutic agents approved by the FDA are acetylcholine esterase (AChE) inhibitors (e.g., donepezil), which enhance cholinergic neurotransmission by hindering the breakdown of acetylcholine. This approach, while providing modest clinical gains, is not believed to retard the progression of the underlying disease. Hence, there is a need to find a suitable drug that attacks the disease at its pathogenesis.

We have reviewed findings which suggest that metal complexing agents may have therapeutic benefits in AD. In the last few years, chelation therapy has become increasingly promoted as a therapy for AD, both by individual medical practitioners and by lay groups and even at internet web sites. However, the growing practice of intravenous infusions of EDTA is based upon largely unscientific interpretations of the neurochemical problems in AD, and can lead to systemic metal ion depletion.

One previous clinical trial of the chelator compound DFO was reported to significantly arrest the progression of the disease.<sup>59</sup> Although the DFO trial was thought to target Al(III), it is possible that the beneficial effect of the treatment was due to chelation of Fe(III), Cu(II), and Zn(II). Indeed, the authors reported verbally (International Conference on Alzheimer's Disease, Padua, 1992) that postmortem metal analysis on brain tissue of study-subjects indicated that although aluminum levels were lower than placebo controls, zinc and iron levels were also decreased in the brains of DFO-treated subjects. This is because, like all chelators, DFO has only a relative selectivity for aluminum, and will also complex with zinc, copper, and iron.<sup>60</sup> Although the results of Crapper-McLachlan and colleagues<sup>59</sup> have not yet been reproduced, further consideration of the removal of zinc, copper, and iron from brain A $\beta$  collections as a therapeutic maneuver seems warranted (FIG. 1). DFO is a charged molecule that does not easily penetrate the blood-brain barrier and is easily degraded after it is administered.<sup>52</sup> Further clinical research into the effects of DFO may have been met with diminished enthusiasm, since the administration of DFO is associated with discouraging difficulties including the nonspecific problems of systemic metal ion depletion (e.g., anemia), and the problem of administration of a twice-daily, painful intramuscular injection.

We propose that the metal binding sites on A $\beta$  may provide an appropriate drug target for rational drug design. Although the 3-D structure of A $\beta$  is unknown, our data indicate that the redox active metal binding site on the protein is subject to steric principles, and therefore small compounds with great specificity for this site may be developed. The principles of pharmacotherapeutic molecule complexing a metal-binding site on a protein target is actually well developed in pharmacology. Several well-known antibiotic, anticonvulsive, antitumor, and antiinflammatory drugs (TABLE 2) exert their pharmacological action by interacting with the Cu-, Zn-, or Fe-active site of their target protein. Disulfiram, for example, blocks enzyme activity by chelating the zinc-catalytic site of alcohol dehydrogenase.<sup>61</sup> Nonsteroidal antiinflammatory drugs (NSAIDs) such as aspirin, diflunisal, ibuprofen, naproxen sodium, indomethacin, *D*-penicillamine, etc., block the heme-iron catalytic site on



TABLE 2. A list of drugs that possess chelating properties

Usage	Drug name	Metal Chelate	Reference
Antiinflammatory; Aanalgesic	Aspirin, indomethacin, <i>d</i> -penicillamine, ibuprofen	copper, iron, zinc	62,71-73
Antibiotic; antitumor; sedative	Bleomycin, ethambutol, thalidomide	copper, iron, zinc	74-76
Antioxidant; dietary supplement	$\alpha$ -lipoic acid	zinc, copper, manganese	77
Anticonvulsant	Valproate sodium, phenytoin	copper, selenium, zinc	78,79
Alcohol abuse	Tetraethyl thiuram disulfide or diethyl dithiocarbamate (disulfiram/antabuse)	copper, zinc	53

cyclooxygenase/arachidonic acid pathway.<sup>62</sup> Intriguingly, the use of these drugs has also been reported to reduce the epidemiological risk for AD,<sup>63</sup> but their therapeutic value is still uncertain (see also Progress Report on Alzheimer's Disease, NIA/NIH publication).

### CONCLUDING REMARKS

Our current findings indicate that an ideal therapeutic drug to dissolve A $\beta$  amyloid would involve a compound that is relatively selective for Cu(I), Zn(II), and possibly Fe(III), but does not sequester Mg(II) or Ca(II), and that coordinates metal ions in the cerebral amyloid mass but not systemically. Charged species cannot diffuse through biological membranes and thus are confined to the tissue compartment where they were administered. Electrically neutral and nonpolar molecules are ideal chelators, since they are best absorbed across the gastrointestinal tract and achieve a broad distribution throughout various tissues. Finally, tissue and target selectivity of the chelator-drug is essential in order to prevent other biologically important metal ions from becoming systemically depleted during therapy.

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